

DENSITY-DEPENDENT SELECTION ON GAMETE TRAITS IN THREE CONGENERIC SEA URCHINS

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Abstract. Because male distribution and abundance strongly influence female fertilization success, density-dependent selection might act on gamete traits to maximize reproductive success. Intra- and interspecific variation in female fertilization success was investigated in the sea urchins *Strongylocentrotus purpuratus*, *S. franciscanus*, and *S. droebachiensis*. Male and female urchins of each species were induced to spawn within 25-m² plots. The percentage of eggs fertilized, along with six covariates (male density, male–female distance, number of males, surge velocity, advection, and water depth), was measured for each spawning event. *S. purpuratus* had the highest levels of fertilization (94%), followed by *S. franciscanus* (64%) and *S. droebachiensis* (23%). The species with the highest densities, most males, and closest neighbors had the highest levels of fertilization. Depth and surge were not significant factors, but advection was associated with fertilization negatively in *S. purpuratus* and positively in *S. droebachiensis*. This interaction is probably caused by the higher degree of clumping in *S. purpuratus* than in *S. droebachiensis*. An analysis of covariance indicates that the rank order of least-squares means of percentage fertilization for these three species is the opposite of that for the uncorrected species means. *S. droebachiensis* performs best and *S. purpuratus* performs worst, when conditions are held equal. These differences in performance can best be explained by gamete traits. Additional experiments determined (1) that the 25-m² plot size is the appropriate scale for investigation of patterns of fertilization in *S. franciscanus*, the species with the largest contiguous populations; (2) that *S. purpuratus*, the species commonly found in the intertidal and subtidal zones, had similarly high levels of fertilization in the two locations; and (3) that fertilization success in the experimental plots was similar to that during natural spawning events. These results are consistent with density-dependent selection on gamete traits. Gametes of the species that naturally occurs at the lowest population density and has the lowest natural fertilization rates have attributes that cause them to perform the best of the three under conditions of sperm limitation, and those of the species that occurs at the highest densities and has the highest levels of natural fertilization perform most efficiently under conditions of sperm competition. Historical demographic conditions appear to have resulted in selection on gamete traits to maximize reproductive success under conditions of either sperm limitation or sperm competition.

Key words: Allee effects; density-dependent selection; echinoid; ecology; fertilization; egg number and size; life history strategies; sperm competition; sperm limitation.

INTRODUCTION

The notion that persistent differences in population density, or in how a population is regulated, can influence selection on life history traits remains at the heart of the interface between population ecology and population genetics (see e.g., MacArthur and Wilson 1967, Mueller 1997, Leips and Travis 1999, Leips et al. 2000). However, the substantial efforts to document density-dependent selection have focused on postzygotic traits. Density-dependent selection on gametic traits may be an important process in the wide diversity of organisms with external fertilization. In external fertilizers, population density strongly influences fertilization success. Eggs spawned in high-density populations are less likely to suffer from sperm limitation,

and sperm are more likely to compete. Conversely, in low-density populations, success of both sperm and eggs is likely to be limited by gamete encounters (reviewed by Levitan 1995, 1998a, Yund 2000). These density-dependent gamete interactions are likely to result in different selective pressures for both gametes and adults (Levitan 1998a).

Unfortunately most studies of fertilization ecology have concentrated on how population parameters influence female fertilization success without considering variation in gamete traits (reviewed by Levitan 1995, 1998a, Levitan and Petersen 1995, Yund 2000) or have studied the influence of gamete traits without considering variation in population attributes (Levitan 1993, 1996a, b, 1998a, b, 2000, Thomas 1994a, b, Mead and Denny 1995, Podolsky and Strathmann 1996, Styan 1998, Thomas et al. 1999, Marshall et al. 2000). The first line of research demonstrates that female fertilization success is highly sensitive to variation in the

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distribution and abundance of spawning males. The second indicates that intra- and interspecific differences in gamete traits result in predictable differences in fertilization performance in the laboratory and field, when population parameters are held equal. The rationale for combining these two approaches is that gamete traits associated with fertilization have evolved not in isolation but within the context of the demographic and flow regimes that have been documented to influence levels of fertilization (Levitan 1993, Lasker et al. 1996).

Gamete traits are predicted to evolve differently under different degrees of sperm limitation or competition. Under sperm limitation, eggs are predicted to present larger target size and sperm receptivity, and sperm are predicted to have greater longevity. Under sperm competition, eggs are predicted to be smaller and more numerous and to be more selective, and sperm are predicted to be faster (Levitan 1993). Previous research has documented how these gamete traits perform under various conditions of sperm availability (Levitan 1993, 1996a, 1998b, 2000). All previous experiments on external fertilizers have tested gamete performance under uniform conditions in the laboratory or with a single point source of sperm in the field. What remains unknown is how species differences in demography and the physical environment may influence fertilization and whether population density, gametic performance, and gametic traits are correlated.

Here, I experimentally investigate levels of female fertilization success in three congeneric sea urchins spawning at natural densities in their natural habitat. Intra- and interspecific variation in fertilization success is correlated with variation in environmental, population, individual, and gamete-level factors. These three species differ in gamete traits, population density, and the degree of sperm limitation they experience. Their gametes appear to be adapted to different positions on this gradient from sperm limitation to sperm competition.

The sea urchins

Off the coast of British Columbia, *Strongylocentrotus droebachiensis*, *S. franciscanus*, and *S. purpuratus* inhabit the shallow subtidal. *S. franciscanus* and *S. purpuratus* range from southeast Alaska to Baja California; the latter generally has a shallower distribution extending into the low intertidal (Schroeter 1978). *S. droebachiensis* is a circumpolar species that extends southward along the northwest coast of North America through Washington State. *S. droebachiensis* and *S. purpuratus* reach a maximum size of ~8-cm test diameter (Kramer and Nordin 1978, Schroeter 1978), and *S. franciscanus* grows to 17 cm (Bernard and Miller 1973, Levitan et al. 1992). All three species spawn unfertilized gametes into water in the winter and spring (Strathmann 1987, Levitan 1998a) and undergo meta-

morphosis at similar sizes (Sinervo and McEdward 1988).

These species differ in distribution and abundance along the northwest coast of North America. *S. purpuratus* tends to be patchily distributed and packed densely into crevices along high-energy shorelines in the shallow subtidal or low intertidal (Schroeter 1978, Levitan 1998a). *S. franciscanus* is more uniformly abundant, covering large expanses of rocky subtidal regions over a large range of densities (Schroeter 1978, Levitan 1998a). *S. droebachiensis* tends to occur at much lower densities, sporadically co-occurring with the other two species (Kramer and Nordin 1978, Rumrill 1987, Waddell et al. 1997, Levitan 1998a). Mean intraspecific nearest-neighbor distances for the three species in Barkley Sound, British Columbia, are 19, 40, and 134 cm for *S. purpuratus*, *S. franciscanus*, and *S. droebachiensis*, respectively (Levitan 1998a). There is a general debate over historical sea urchin population densities in coastal regions prior to human removal of sea urchin predators (e.g., East Coast North America, Mann and Breen 1972, Miller 1985, Elner and Vadas 1990; Caribbean, Hay 1984, Levitan 1992, Jackson 1997) and specifically about the consequences of eliminating sea otter populations along the northwest coast of North America (Estes and Palmisano 1974, Foster 1990, Estes and Duggins 1995, Estes et al. 1998). However, the degree of local clumping of these three species may be more important to fertilization than is overall abundance (see *Results*).

These three species show marked differences in gamete morphology and fertilization performance. *S. purpuratus* has the smallest eggs and the fastest but shortest-lived sperm of the three species. *S. droebachiensis* eggs are five- to sixfold larger in volume than those of *S. purpuratus*, and its sperm are the slowest but longest lived of the three. *S. franciscanus* has intermediate egg size, sperm velocity, and sperm longevity (Levitan 1993). Laboratory studies indicate that within and among the three species, larger eggs are preferentially fertilized under conditions of sperm limitation (Levitan 1993, 1996a). Field studies indicate that, when eggs and sperm are released synchronously and in close proximity, the three species do not differ significantly in fertilization rate, but when sperm were dispersed before contact with eggs, the rank order of fertilization performance matched expectations based on egg size difference; species with larger eggs did better under conditions of sperm limitation (Levitan 1998a).

No studies have addressed the influence of sperm swimming ability on fertilization rates in these three species, but in the sea urchin *Lytechinus variegatus*, males with faster sperm had higher fertilization rates than did males with slower sperm, but sperm velocity was negatively correlated with sperm longevity (Levitan 2000). Fast sperm may increase the probability that a particular male will fertilize an egg when sperm are competing and eggs are quickly fertilized. Sperm lon-

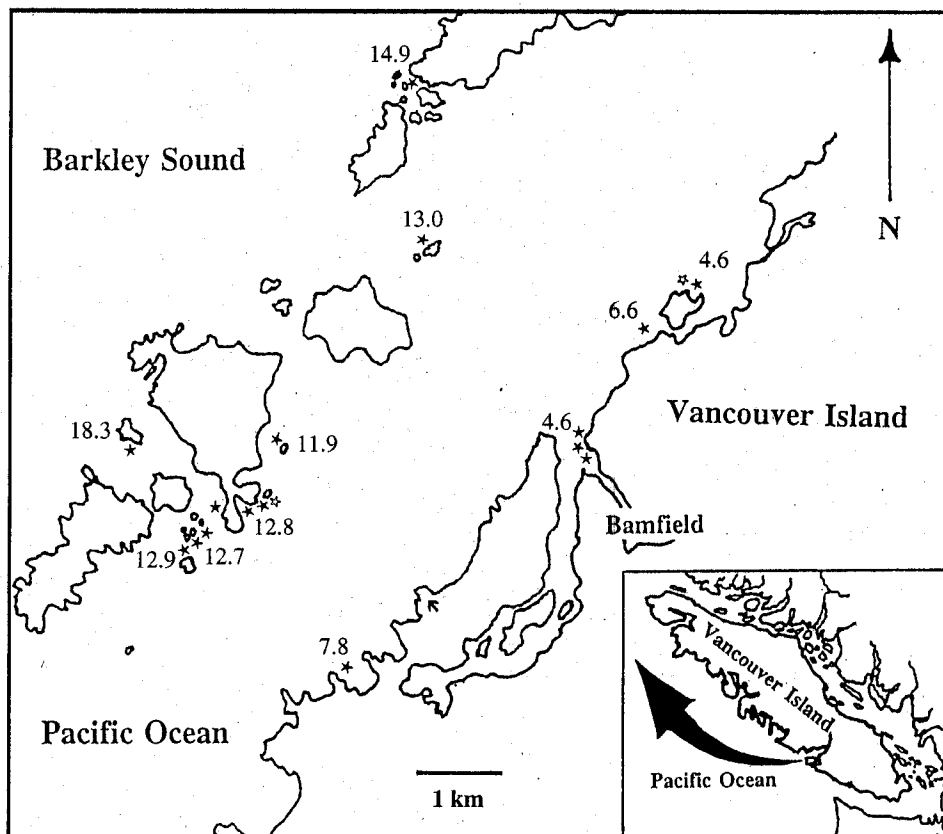


FIG. 1. Map of Barkley Sound, British Columbia, Canada. Each of the 66 spawning events studied was at a unique location, but all were clustered around the star symbols (59 25-m² plots, five low-density *S. purpuratus* experiments, and two 100-m² *S. franciscanus* experiments). The two natural spawning events are indicated by open star symbols. An arrow indicates the location of the tidepool experiments. Numbers indicate mean surge velocity (cm/s), during the experiments, for each area.

gevity may be more important when sperm are limiting and sperm must maintain viability until reaching widely dispersed eggs (Levitan 1993, 1998a, b). The negative correlation between sperm velocity and longevity among the three *Strongylocentrotus* congeners and within *Lytechinus variegatus* suggests a within-spermatozoon energetic trade-off may reflect selection for success under either sperm limitation or competition. Together, these studies suggest that the gamete attributes of *S. droebachiensis* increase the likelihood of fertilization when sperm are limiting and that those of *S. purpuratus* increase that likelihood when sperm are not limiting and are presumably competing.

METHODS

Subtidal mass spawning experiment

Research was conducted off the west coast of Vancouver Island, Canada, in the winters and springs of 1995–1999. Experiments were conducted throughout the Deer Island Group and the southwestern coast of Barkley Sound (Fig. 1). At each of 59 locations a 5 × 5 m plot, subdivided into 1-m² quadrats, was haphazardly chosen with the restriction that the plot had to

contain at least two individuals of the same species. An S4 InterOcean current meter (InterOcean Systems, San Diego, California), programmed to record current velocity, current direction, and depth at 0.5-s intervals, was placed 0.5 m off the substratum 5 m from the study site. Only one species was examined during each induced spawning event. Every urchin of the focal species within the plot was picked up, injected with 0.55 mol/L KCl to induce spawning, and immediately returned to the exact original location. Divers carefully moved backwards (fins first) along the bottom so that any diver-induced turbulence preceded the injection of urchins rather than stirring up the water column over the urchins after they started to spawn. Spawning generally started within a minute or two after KCl injection. After 30 min of spawning, I mapped the positions of all individuals within each 1-m² subdivision and recorded whether the individual was male, female, or nonspawning. I collected a sample of eggs from the water directly above each spawning female for 5–10 s. The eggs were collected with a pump connected to one of 12 1.5-L chambers, each containing a 30-μm filter (see Levitan 1996a and 1998b for pump design). Im-

mediately after the egg sample was taken, the intake hose was moved to a point at least 5 m away from any spawning urchin and rinsed with seawater for 1 min to remove any residual sperm. The pump could sample a maximum of 12 females from each spawning event. In the rare instances when more than 12 females spawned, 12 females were chosen haphazardly for sampling.

The patchy distribution of *S. purpuratus* was such that all individuals were usually situated in one area of the 25-m² plot. The nearest conspecifics to these localized patches were generally >10 m away. In the trials using *S. droebachiensis*, a few individuals were occasionally found outside the 25-m² plot. These individuals were also mapped and injected with KCl as long as they were within 5 m of the plot. As with *S. purpuratus*, generally, additional individuals would be >10 m from this patch. Previous research (Pennington 1985) has indicated that males >5 m from a female are unlikely to influence fertilization success. For these two species the size of the spawning population examined was a function of the natural breaks in distribution of these species. In contrast, *Strongylocentrotus franciscanus* populations often cover hundreds to thousands of square meters. For this species the 25-m² plot represented a small subsample of the local population.

Egg samples were left to incubate at ambient seawater temperatures (8–12°C) for three hours. Then samples of 100–400 eggs per female were investigated for evidence of fertilization (raised fertilization envelope or later developmental stages). Previous studies have demonstrated that the pump used to collect eggs does not cause unfertilized eggs to raise their fertilization membranes or trigger development (Levitan 1998b). Average female fertilization success was calculated from all the spawning females in a spawning event.

Female fertilization success was then correlated with population (number of spawning males, average nearest male distance to females, male density) and physical (surge velocity, advection, and depth) parameters. Male density was calculated as the number of males divided by the area occupied by the sea urchins. This area was defined as the circle with a diameter equal to the maximum distance between spawning individuals. This definition of area was used because at times all the sea urchins were clumped in a subsection of the full 5 × 5 m plot and at others, occasionally with *S. droebachiensis*, were scattered over a larger area. Surge velocity was calculated from the S4 current meter as the mean of the absolute values of the current velocity calculated at 0.5-s intervals over the duration of the experiment. This value is highly correlated with the rate at which a sperm cloud expands (and becomes diluted) at these study sites and provides an index of turbulence (Levitan 1998b). Advection was estimated using the "progressive vector" output from the S4 current meter. The progressive vector is the distance and direction a particle of water would move over the time period of the experiment if flow conditions were spa-

tially homogenous. Advection was calculated as the straight-line distance of the progressive vector over the full experimental time, divided by that time. The result provides an estimate of the interval during which a water mass containing sperm would probably reside over a spawning aggregation and the rate at which it would move downstream.

Data were transformed so that female fertilization success (logistic transformation) was linearly related to the three population (natural logarithmic transformation) and three physical parameters. To test for species differences, I subjected the transformed data to analyses that both did not (ANOVA) and did (ANCOVA) account for these six covariates.

Modifications of mass spawning experiment

I conducted two additional experimental treatments to address two aspects of species differences in distribution. In the first, before inducing the sea urchins to spawn, I manipulated *S. purpuratus*, the species that normally lives in a clumped distribution (Levitan 1998b and see *Results*), to produce the lower densities typical of *S. droebachiensis*, by haphazardly placing urchins throughout the full 5 × 5 m plot at low densities. In the second, to determine whether the much larger spatial extent of *S. franciscanus* populations made estimates of fertilization based on the relatively small 5 × 5 m plots inappropriate for this species, I induced all *S. franciscanus* within a 10 × 10 m plot to spawn, then sampled only the females within the center 5 × 5 m section.

Data from natural spawning events

On two occasions, sea urchins spawned naturally during the experimental trial. On one occasion (4 April 1995), the event started after the plot was laid out. Density of *S. franciscanus* was high, and the other two species absent. On the other occasion (9 March 1998), in a location where *S. franciscanus* density was also high, the two other species were present and were also spawning. This event was in progress as divers entered the water. These natural spawns did not appear to be caused by the presence of the divers and were initiated before any animals were injected with KCl. In both cases the spatial extent of the spawning event was larger than the 5 × 5 m plot. Data were collected in a manner similar to that of the induced spawning events.

Intertidal experiments

In spring 1996, I assessed fertilization success of *S. purpuratus* in 10 tidepools. At low tide, when the tidepools were isolated from the ocean, I picked up each urchin, injected it with KCl, and returned it to its original location; I also mapped the dimensions of the tidepool and the position of each urchin. After 30 min of spawning, I collected a sample of eggs with a 10-mL disposable pipette. Thereafter I collected samples of eggs from each female at 1-h intervals before the ap-

proaching waves reached the tidepool and then at 10- to 15-min intervals until the tidepool was completely awash and sampling became impossible. In addition, the timing of each wave reaching the tidepool was recorded until the tidepool was completely awash.

Potential artifacts of data collection

The two methods of collecting fertilization data have been to place eggs in sperm-permeable containers (Yund 1990, Levitan 1991, Levitan et al. 1992, Levitan and Young 1995, Wahle and Peckham 1999) and to collect free-drifting eggs (Levitan 1991, 1996a, 1998b, Petersen 1991, Babcock et al. 1992, 1994, 2000, Babcock and Mundy 1992, Oliver and Babcock 1992, Petersen et al. 1992, Warner et al. 1995, Hamel and Mercier 1996, Lasker et al. 1996, Coma and Lasker 1997, Marconato et al. 1997). Placing eggs in containers returns data on the cumulative levels of fertilization that result from sperm able to drift or swim through the mesh holding the eggs. This method results in an overestimate of fertilization if eggs would naturally drift into a large expanse of seawater but would underestimate fertilization if the eggs were held in a low-density population but might naturally drift over a mass of spawning males.

Collecting free-drifting eggs returns cumulative fertilization over a much shorter period of time, as eggs quickly drift to a degree where they are difficult for divers to track or collect. This method would tend to underestimate fertilization success if eggs drifted along an expanse of spawning males rather than up into the water column (Lasker et al. 1996, Coma and Lasker 1997).

The steep bottom topography of Barkley Sound is such that the probability is relatively high that eggs would drift rapidly into the open ocean and not over additional spawning males. The probability of fertilization is highest at the point of release and probably diminishes rapidly over time. Holding eggs in containers at this point of release would probably overestimate levels of fertilization under these conditions. In the present study some eggs might have been collected before they had a chance of additional fertilization, but this artifact is probably much less than that resulting from holding eggs at the site of release.

RESULTS

Mass spawning experiment

Overall, all three urchin species differed significantly in average female fertilization success in their natural habitats and natural population distributions and abundances (ANOVA $F = 14.09$, $df = 2, 56$, $P < 0.0001$; all pairwise comparisons significantly different by Tukey's studentized range test). *S. purpuratus* had the highest level of fertilization (94% backtransformed mean), followed by *S. franciscanus* (64%) and *S. droebachiensis* (23%). When the manipulated *S. purpuratus*

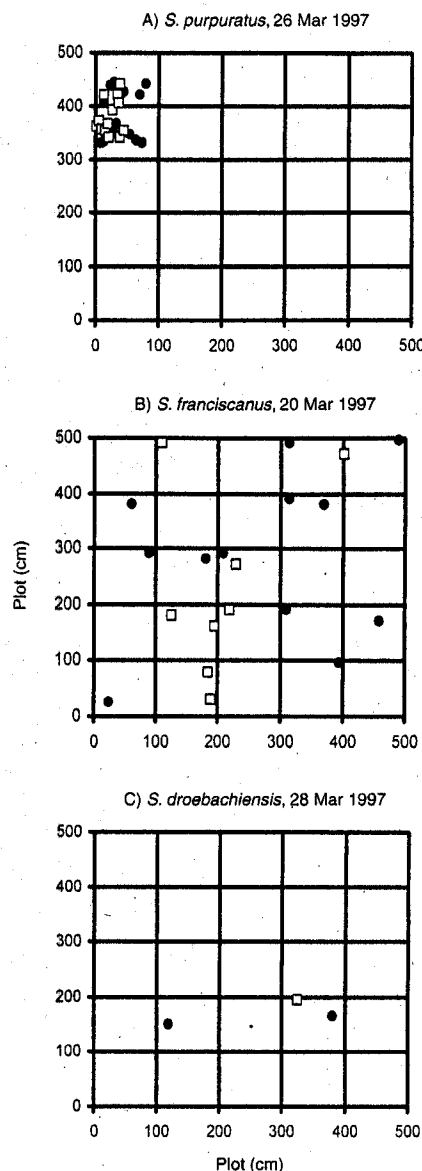


FIG. 2. Representative distributions of spawning individuals from experimental mass spawning experiment. Plots are 5×5 m areas subdivided into 1-m^2 sections: (A) *S. purpuratus*; (B) *S. franciscanus*; (C) *S. droebachiensis*. Solid circles represent positions of spawning females; open squares represent positions of spawning males.

data were added to this analysis, the results were still significant, and the rank order of female fertilization success remained the same ($P < 0.0001$). Representative mapped sites of the three species illustrate differences in the distribution and abundance of the three species (Fig. 2A, B, C). Differences in fertilization rates were correlated to varying extents with the number of spawning males (Fig. 3A), the distance to a spawning male (Fig. 3B), male density (Fig. 3C), surge velocity (Fig. 3D), advection (Fig. 3E), and water depth (Fig. 3F).

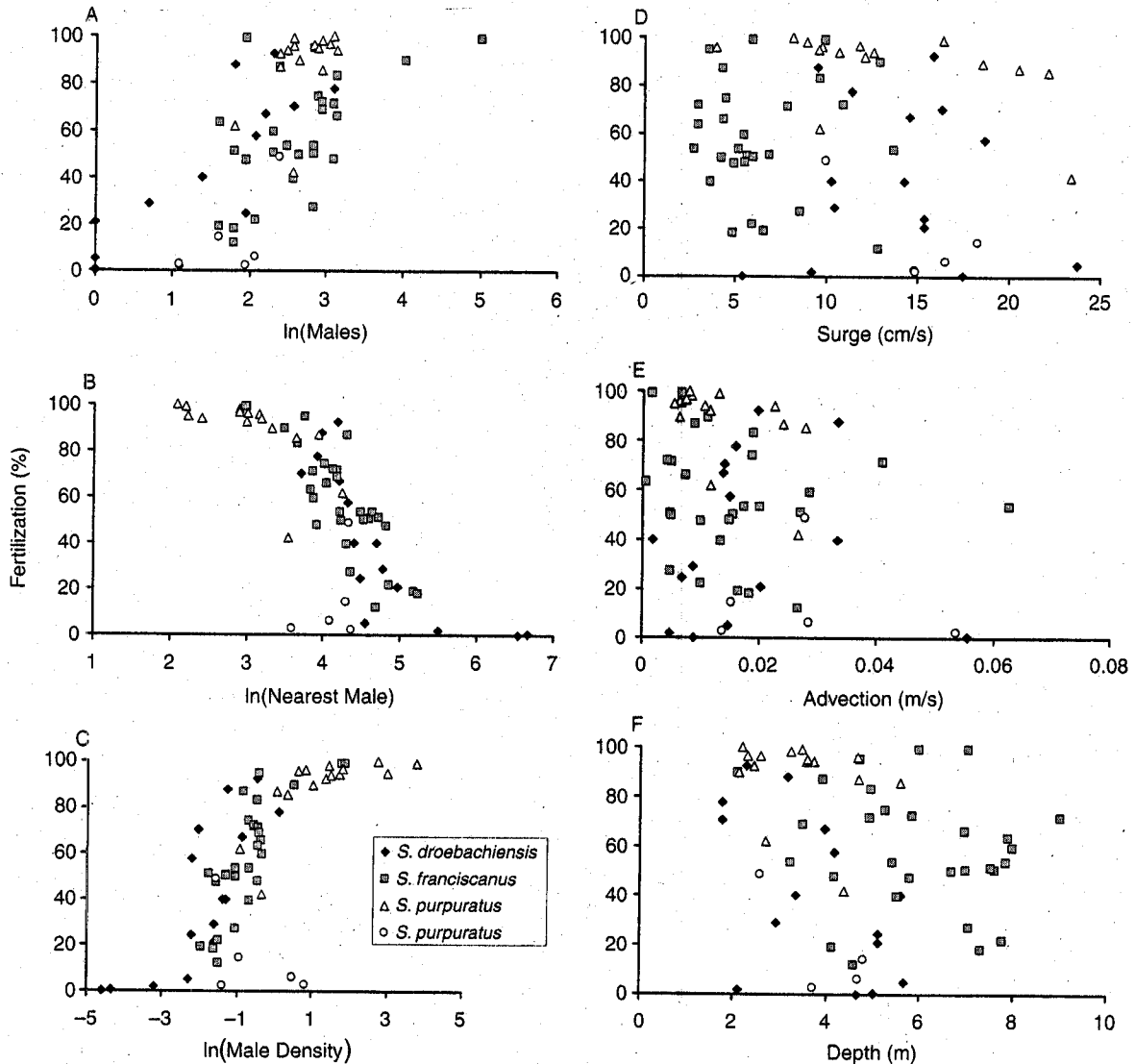


FIG. 3. The mean female fertilization success for each spawning event as a function of three male and three environmental variables: (A) number of males, (B) mean spawning-female-to-male distance (measured in cm), (C) male density (measured in no./m²), (D) surge velocity, (E) advection, and (F) water depth. Raw data are plotted; values were transformed to insure that the effects were linear for the statistical tests. The open-circle symbols represent the manipulated, low-density, *S. purpuratus* trials.

To examine species differences in fertilization rates as a function of these six covariates, I used the full data set with the manipulated *S. purpuratus* density treatments so that a larger range of population values overlapped among the three species. When the covariates were added to the model, the adjusted mean species values changed dramatically. The ANCOVA tested the response variable of average female fertilization success (logistic transformed) with a main effect of species and the covariates of male density (\ln -transformed), the number of spawning males (\ln -transformed), mean nearest spawning male neighbor (\ln -transformed), surge velocity, advection, and water depth. This analysis indicated a significant main effect (species), two

significant covariates (average male-female distance and male number), and two significant interactions with the main effect (male number and advection) (Table 1). Most interestingly, the least-squares means for the three species (the expected species means with all covariates at their mean value) were in a rank order opposite to that of the population means, which did not consider the covariates (Fig. 4). The least-squares mean was highest for *S. droebachiensis* (76%), followed by *S. franciscanus* (71%) and *S. purpuratus* (37%). The species with the lowest natural levels of fertilization has the best-performing gametes, and the species with the highest natural levels of fertilization had the worst, when the effects of the covariates were held at average

TABLE 1. ANCOVA results for species differences in fertilization rates of sea urchins (ANCOVA conducted with interaction terms).

Source	df	Sum of squares	Mean square	F	P
Model	12	361.010	30.084	26.50	<0.0001
Error	51	57.894	1.135		
Total	63	418.903			
Species	2	12.652	6.326	5.57	0.0065
No. males	1	17.898	17.898	15.77	0.0002
Nearest male	1	4.954	4.954	13.17	0.0007
Male density	1	0.554	0.554	0.49	0.4879
Advection	1	0.040	0.040	0.04	0.8515
Depth	1	0.340	0.340	0.30	0.5865
Surge	1	0.180	0.180	0.16	0.6922
No. male \times Species	2	13.992	6.996	6.16	0.0040
Advection \times Species	2	8.422	4.211	3.71	0.0313

Notes: The table presents results from analysis of covariance testing species differences in mean fertilization success (logistic transformation) as the main effect, with the number of spawning males (ln-transformed), average distance between spawning female and its nearest spawning males (cm; ln-transformed), spawning-male density (no./m²; ln-transformed), advection (m/s), water depth (m), and surge velocity (cm/s) as covariates. Significant interactions of male number and advection are presented. Type III sums of squares are reported. Total $R^2 = 0.862$.

values. An analysis without the significant interaction terms produces the same significant covariates, main effect (Table 2) and rank order of least-squares means (*S. droebachiensis*, 79%; *S. franciscanus*, 65%; *S. purpuratus*, 40%). The significant interaction involving the number of males is a difference in the steepness of the positive relationship between the number of males and fertilization success. Although all species have high fertilization rates when many males spawn, *S. purpuratus* does relatively poorly when only a few individuals spawn (Fig. 3A). This result was especially evident in the *S. purpuratus* populations that were induced to spawn at densities typical of *S. droebachiensis*. At similarly low numbers of spawning males, the *S. purpuratus*

females had between 20% and 40% lower fertilization success than did *S. droebachiensis*. The significant interaction involving the level of advection is a difference in the direction of the slopes. There is a positive relationship between advection and fertilization success in *S. droebachiensis* and a negative one in *S. purpuratus* (Fig. 3E).

Because there were two significant interaction terms, stepwise regressions on the fertilization data were conducted independently on the three species. The results indicate that average nearest-male distance was a significant factor in all three species, together with either the number of spawning males (*S. purpuratus*) or the male density (*S. droebachiensis* and *S. franciscanus*). The rate of advection was a significant factor for *S. droebachiensis* and *S. purpuratus* (Table 3). Interestingly, the relationship between advection and fertilization success was negative for *S. purpuratus*, the most clumped species, and positive for *S. droebachiensis*, the most dispersed species. Advection had a nonsignificant influence on fertilization for *S. franciscanus*, the species with intermediate nearest-neighbor distances but the most spatially extensive populations. It is worth noting that, although average nearest-neighbor distances explained most of the variation in average fertilization success, variation was large in the relationship between the individual distance of a female to a male and her fertilization success (Fig. 5), probably because of directional differences in flow.

Population density and number of males were positively related, and population density and the distance between males and females were negatively related (Table 4). Because population density is defined by the spatial extent of the sea urchins, rather than by the arbitrary 25-m² plot, these correlations, although expected, are not mathematical certainties. Population density is also negatively correlated with both depth and advection.

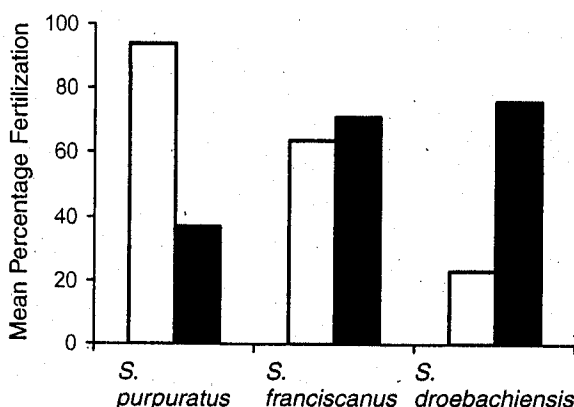


FIG. 4. Histogram of mean species values of fertilization success with and without consideration of the covariates. Values calculated without consideration of the covariates are the actual mean levels of fertilization among these three species in these experiments (open bars). The values for which the covariates were considered (least-squares means) are estimates of the level of fertilization if all the covariates are held equal and reflect species performance under uniform conditions (solid bars).

TABLE 2. ANCOVA results for species differences in fertilization rates of sea urchins (analysis conducted without interaction terms).

Source	df	Sum of squares	Mean square	F	P
Model	8	336.883	42.110	28.24	<0.0001
Error	55	82.020	1.491		
Total	63	418.903			
Species	2	13.741	6.870	4.61	0.0141
No. males	1	6.856	6.856	4.60	0.0365
Nearest male	1	17.580	17.580	11.79	0.0011
Male density	1	2.593	2.593	1.74	0.1928
Advection	1	0.029	0.029	0.02	0.8897
Depth	1	0.118	0.118	0.08	0.7800
Surge	1	1.813	1.813	1.22	0.2750

Notes: The table presents results from analysis of covariance testing species differences in mean fertilization success (logistic transformation) as the main effect, with the number of spawning males (ln-transformed), average distance between spawning female and its nearest spawning male (cm; ln-transformed), spawning male density (no./m²; ln-transformed), advection (m/s), water depth (m), and surge velocity (cm/s) as covariates. Type III sums of squares are reported; total $R^2 = 0.804$.

Larger scale experiment

The larger scale experimental spawning of *S. franciscanus* involved mapping and injecting hundreds of sea urchins in the 100-m² area. This experiment was conducted once at relatively high population density (1.4 males/m²) and once at relatively low population density (0.36 males/m²). In both cases the fertilization success of females within the inner 5 × 5 m area was predicted by the relationship between male density and fertilization success of the experimental population, calculated from the density of males within the inner 5 × 5 m area (Fig. 6). A fourfold increase in the spatial scale of the mass spawning event did not appear to have any significant effect.

Natural spawning events

When natural spawning events were observed, all the equipment was on hand for conducting a mass spawning experiment. A direct comparison to the experimental spawnings was therefore possible. The first

of the two natural spawning events took place in a small cove on the north side of Dixon Island, a small shallow bay with a boulder and sand substrate. The only sea urchin at this site is *S. franciscanus*. A heavy phytoplankton bloom was present, and at 1340 Pacific Standard Time (PST), shortly after the 5 × 5 m plot was laid out, male urchins were seen to spawn. A survey of several hundred square meters surrounding the plot revealed no obvious differences in the density, distribution, or percentage of spawning animals between the areas inside and outside the plot.

The distribution of the urchins was mapped at 30-min intervals. A two-way ANOVA indicated that nearest-neighbor distances did not change significantly during the five mapped intervals and that spawning and nonspawning sea urchins did not differ in this variable ($P > 0.99$); the sea urchins did not become more aggregated during the spawning event. The average distance between spawning female sea urchins and the nearest spawning male during the spawning event fell

TABLE 3. Stepwise regression testing the influence of number of spawning males, average distance between spawning males and females, spawning male density, advection, water depth, and surge velocity on fertilization success independently for each species of sea urchin.

Step	Variable	Parameter estimate	Partial R^2	Model R^2	F	P
<i>S. purpuratus</i> (n = 20 spawning events)						
1	No. males	2.679	0.6505	0.6505	33.50	<0.0001
2	Nearest male	-1.240	0.1568	0.8073	13.83	0.0017
3	Advection	-64.043	0.0488	0.8561	5.43	0.0332
<i>S. franciscanus</i> (n = 29 spawning events)						
1	Male density	0.932	0.7678	0.7678	89.29	<0.0001
2	Nearest male	-1.096	0.0224	0.7903	2.78	0.1074
<i>S. droebachiensis</i> (n = 15 spawning events)						
1	Nearest male	-2.381	0.8410	0.8410	68.76	<0.0001
2	Advection	51.029	0.0615	0.9025	7.57	0.0176
3	Male density	0.858	0.0328	0.9353	5.58	0.0377

Notes: The criterion for including a variable in the model is significance at the 0.15 level. Note that the parameter estimate for advection is negative for *S. purpuratus*, nonsignificant for *S. franciscanus*, and positive for *S. droebachiensis*. Data transformations are as in Table 2.

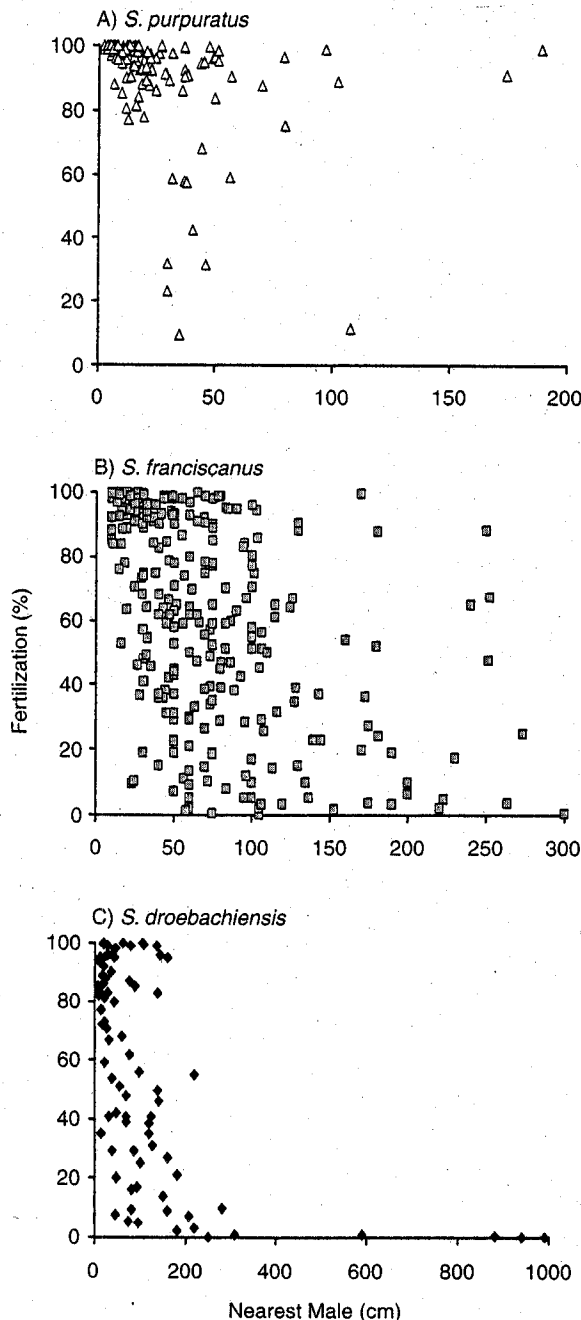


FIG. 5. Percentage of fertilization of each female sea urchin across all spawning events plotted as a function of the distance between that female and the closest spawning male sea urchin.

within the range predicted by the experimental data (Fig. 7), so aggregation was not higher during the natural spawning event than during the experimental ones.

The male urchins continued to spawn; then at 1500, two females were observed spawning, and egg samples were collected from each female. At this peak spawning time, 17 males and 2 females out of 64 adult sea urchins within the plot (30%) were spawning. Female fertil-

ization success was high for the two females (89.9% and 99.8%), as predicted from the density of spawning male urchins and the experimental spawning data (Fig. 8A).

At 1600, only one male continued to spawn within the plot, and a brief survey found no additional urchins spawning outside the plot. Within several minutes after the cessation of spawning, any gametes that had collected on the aboral surfaces of the urchins had been wafted up into the water column. Flow at this site is slight in general and on this day averaged a fairly typical 3.5 cm/s (Fig. 1). There was little wave action, and the tide was falling.

The second spontaneous spawning event was near a rock islet off Diana Island. It took place after the first long sunny period (about five days) of the winter, and the water was cloudy with a dense phytoplankton bloom. At this location, a rocky substrate extends from the intertidal zone down to ~10 m, where the substratum changes to sand. *S. franciscanus* occupies the entire rocky subtidal in a band surrounding the islet. *S. purpuratus* is patchily distributed on the exposed side of the islet in the shallow subtidal zone (down to ~5 m). *S. droebachiensis* was uncommon throughout the rocky subtidal band. Spawning of all three species was in progress when the divers entered the water at 1530 PST, and the sea urchins were still spawning at the end of the dive at 1645. The 5 × 5 m plot was laid out in a haphazard location that contained 188 *S. franciscanus* and 8 *S. purpuratus*. A swimming survey revealed that the spawning event continued for at least 50 m in the downstream direction (southeast) but ended at the upstream end at a discontinuity in the rocky substrate caused by a large crevice. Within the area of spawning, no difference was apparent in the proportion of spawning animals or density of animals within and outside the plot. During the swimming survey, I located three *S. droebachiensis* urchins. Overall, 44% of the sea urchins were spawning, and 85% of the spawning sea urchins were male (Table 5). There was a significant deviation from a 1:1 spawning sex ratio (G test, $P < 0.001$). In addition to the sea urchins, the holothurian *Parastichopus californicus* was observed to spawn.

Egg samples were collected from eight female *S. franciscanus*, which showed an average fertilization of 96.2% (range 86.5–99.6%). Eggs were also collected from the single spawning *S. purpuratus* female; 85.7% of her eggs were fertilized. As in the first natural spawning event, the percentage of eggs fertilized for both species was predicted by the experimental spawning data (Fig. 8A, B). In addition, as in the first observation the distances between spawning females and males were predicted by the experimental data (Fig. 7), indicating that the level of aggregation did not increase during the natural spawning event.

Intertidal experiments

Assays of intertidal fertilization of *S. purpuratus* were conducted in the spring of 1996. Experiments

TABLE 4. Correlations among variables involved in sea urchin fertilization.

Variable	Species†	Density	No. males	Distance	Surge	Advection	Depth
Density	<i>S.p.</i>	1.000					
	<i>S.f.</i>	1.000					
	<i>S.d.</i>	1.000					
No. males	<i>S.p.</i>	0.531	1.000				
	<i>S.f.</i>	0.609	1.000				
	<i>S.d.</i>	0.676	1.000				
Distance	<i>S.p.</i>	-0.913	-0.511	1.000			
	<i>S.f.</i>	-0.921	-0.621	1.000			
	<i>S.d.</i>	-0.859	-0.766	1.000			
Surge	<i>S.p.</i>	-0.281	-0.104	0.381	1.000		
	<i>S.f.</i>	0.140	0.284	-0.103	1.000		
	<i>S.d.</i>	0.085	-0.030	-0.232	1.000		
Advection	<i>S.p.</i>	-0.433	-0.229	0.393	0.765	1.000	
	<i>S.f.</i>	-0.259	-0.174	0.162	0.065	1.000	
	<i>S.d.</i>	-0.173	-0.199	0.297	0.271	1.000	
Depth	<i>S.p.</i>	-0.298	-0.0264	0.310	0.405	0.694	1.000
	<i>S.f.</i>	-0.125	-0.301	0.106	-0.392	0.057	1.000
	<i>S.d.</i>	-0.385	-0.622	0.414	0.384	0.306	1.000

Notes: Data transformed as in Table 2. Unmanipulated *S. purpuratus* data are not included.

† Key to abbreviations: *S.p.*, *Strongylocentrotus purpuratus*; *S.f.*, *Strongylocentrotus franciscanus*; *S.d.*, *Strongylocentrotus droebachiensis*.

were conducted on three days during rising tides, and 10 tidepools were examined. On the assumption of a rectangular shape, tidepools ranged in volume from 0.1 to 5.6 m³. Between one and seven males and one and six females were spawned in each tidepool, and nearest male-to-female distances ranged from 0.15 to 0.5 m. Regardless of the size of the tidepool, the number of males, or the distance between males and females, fertilization reached a peak of near 100% within 1 h of spawning and remained high until the rising tide inundated the pool (Fig. 9). As the waves reached and then splashed into each pool, fertilization rates often declined before sampling the female urchins became too difficult.

Laboratory measures of the distribution of released

eggs from two female *S. purpuratus* indicate that 95% of eggs are released within the first hour after injection with KCl (Fig. 10). If KCl-induced spawning is similar in pattern to natural release, then, for tidepools of the size investigated, the vast majority of released eggs will be fertilized provided that the sea urchins spawn at least one hour before the rising tide reaches the tidepool.

DISCUSSION

The gradient from sperm limitation to sperm competition

The results of this experiment are consistent with density-dependent selection on gamete traits. The spe-

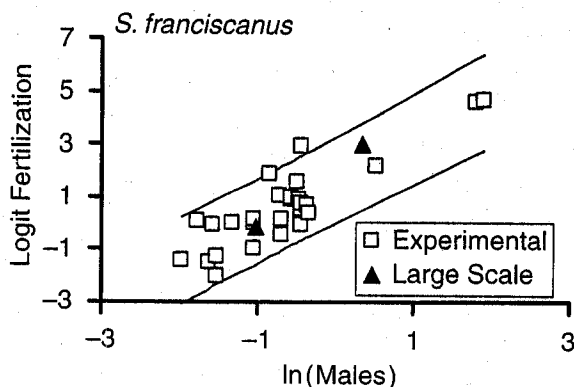


FIG. 6. Mean percentage of eggs fertilized as a function of male density (measured in no./m²) for the regular 5 × 5 m plots and two 10 × 10 m plots. Lines represent the upper and lower 95% confidence intervals on the mean for the regular plots.

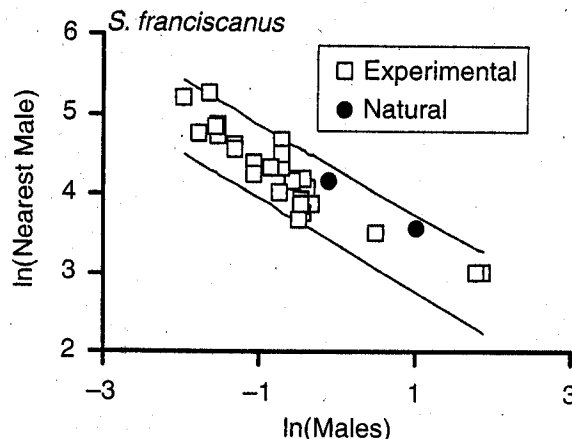


FIG. 7. Nearest-neighbor distances (measured in cm) for both experimental mass spawns and the two natural spawning events (measured in no./m²). Lines represent the upper and lower 95% confidence intervals on the mean for the experimental plots.

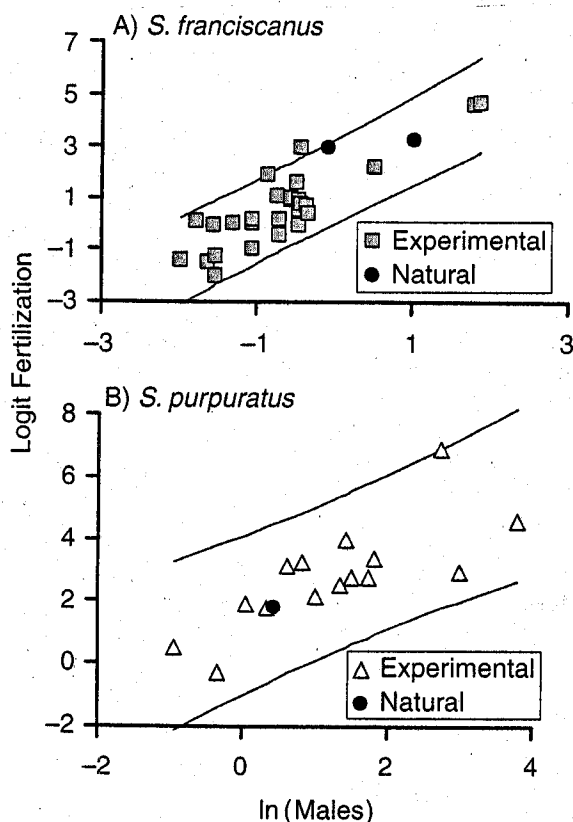


FIG. 8. Mean female fertilization success as a function of male density (measured in no./m²) for experimental and natural mass spawning events for (A) *S. franciscanus* and (B) *S. purpuratus*. Lines represent the upper and lower 95% confidence intervals on the mean for the experimental data.

cies living at the lowest densities has low levels of fertilization and gametes that performed relatively best under conditions of sperm limitation. The species living at the highest densities has the highest levels of fertilization and gametes that performed worst under conditions of sperm limitation. The species living at intermediate densities had intermediate levels of fertilization and gamete performance.

Across all three species, average female fertilization success for a particular spawning event ranged from 0.1% to 99.9%. The ANCOVA explained 86% of this

variation as a function of species differences in reproductive traits and the distribution and abundance of spawning males. *S. purpuratus* has the most clumped distribution (Fig. 3B and Levitan 1998b) and showed an average female fertilization success of 94%. When the average was weighted by population density, so as better to reflect the overall average per-female fertilization success, this value increased to 97%, and when the intertidal estimates were also considered, the predicted average fertilization success for this species in Barkley Sound was even higher. At this level of aggregation and fertilization, males are likely to compete for fertilizations. *S. droebachiensis* is the rarest of the three species in Barkley Sound, and even though it has a patchy distribution, individuals are often 5–10 m from the closest conspecific (Levitan 1998b). This species showed an average fertilization rate of 23% or a weighted average of 60%, and its fertilization is likely to be sperm limited at the densities typically found on the west coast of British Columbia (Kramer and Nordin 1978, Rumrill 1987, Waddell et al. 1997, Levitan 1998b). *S. franciscanus* has intermediate densities but occurs over large spatial expanses over a wide range of population densities. In this species, an average of 64% of eggs were fertilized, and the weighted average was 71%. At various times or places individuals of this species might experience the full spectrum from sperm limitation to sperm competition (Fig. 5).

When adjusted by the effects of the covariates on fertilization, the species rank order of fertilization success reverses; *S. droebachiensis* becomes the most successful, followed by *S. franciscanus* and *S. purpuratus*. The species most subject to conditions of sperm limitation is the most successful at fertilization when population and physical factors are held constant. Conversely, the species that typically has near complete fertilization success showed the poorest performance under conditions of sperm limitation. Although adult characteristics such as spawning rate or reproductive effort might explain such differences, no pattern of adult characteristics is consistent with this rank order of performance. Egg and sperm characteristics do vary, however, and results of this field experiment are consistent with laboratory and field studies of gamete performance (Table 6).

TABLE 5. Percentage of male and female sea urchins that spawned during the natural spawning event of 9 March 1998.

Species	Spawning (no.)		Nonspawners (no.)	Percentage spawners
	Male	Female		
<i>S. purpuratus</i>	4	1	3	62.5
<i>S. franciscanus</i>	73	9	106	43.6
<i>S. droebachiensis</i>	1†	0	2†	33.3

Notes: Numbers for *S. purpuratus* and *S. franciscanus* represent the numbers of spawning and nonspawning sea urchins within the 5 × 5 m plot.

† No *S. droebachiensis* were present in the plot, but a swimming survey revealed three individuals nearby.

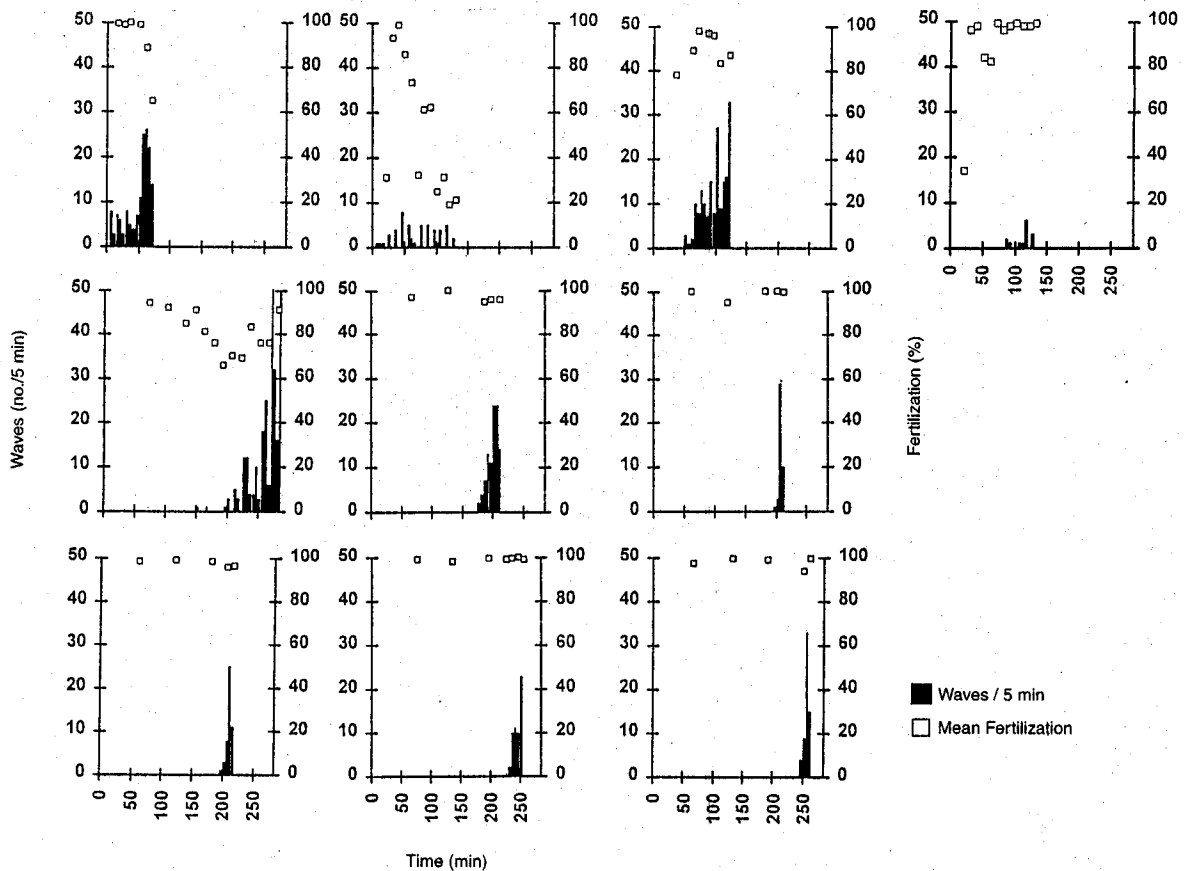


FIG. 9. Number of waves that entered a tidepool within a 5-min interval and the mean fertilization success of females in the tidepool, as a function of time after injection of *S. purpuratus* with KCl. Ten pools were investigated; plots are organized from left to right and top to bottom according to increasing time from injection to the first wave that reached the pool. Data were collected until the tidepool was completely awash.

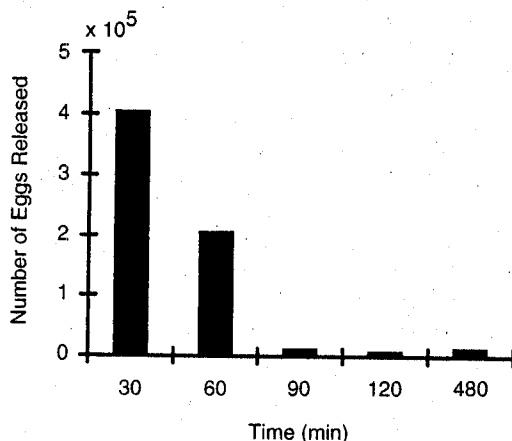


FIG. 10. Number of eggs released by *S. purpuratus*, following injection of KCl. Almost all eggs are released within the first 60 min. Note that y-axis scale numbers indicate hundreds of thousands of eggs.

Laboratory experiments indicate that *S. purpuratus*, the species with the smallest eggs, requires an order of magnitude more sperm to fertilize 50% of a batch of eggs than does *S. droebachiensis*, the species with the largest eggs. *S. franciscanus*, which has intermediate egg size, requires an intermediate amount of sperm to reach this level of fertilization (Levitan 1993). Laboratory studies of intraspecific and intrafemale variation in fertilization also indicate an egg-size effect (Levitan 1996a). Larger egg targets are more frequently struck by sperm and fertilized than are smaller egg targets (Farley and Levitan 2001). In addition to the larger egg size of *S. droebachiensis*, the size-adjusted fertilization rate is three times higher in this species than in the other two (Levitan 1993). This ease of fertilization may also explain why eggs of this species appear to be easily fertilized by *S. purpuratus* and *S. franciscanus* sperm. The eggs of the other two species are much more resistant to hybrid fertilization (*unpublished data*). Perhaps one of the consequences of increasing the likelihood of fertilization under conditions of sperm limitation is an increased risk of hybridization.

Previous field experiments also suggest that *S. droe-*

TABLE 6. Summary of gamete traits, gamete performance, and levels of sperm availability for *S. purpuratus*, *S. franciscanus*, and *S. droebachiensis*.

Variable	<i>S. purpuratus</i>	<i>S. franciscanus</i>	<i>S. droebachiensis</i>
Egg size	Small	Medium	Large
Receptiveness	Low	Low	High
Sperm velocity	Fast	Medium	Slow
Sperm longevity	Low	Medium	High
Gamete performance	Low	Medium	High
Sperm availability	High	Medium	Low

Sources: Egg and sperm traits, Levitan (1993); gamete performance, Levitan (1993) (lab performance) and Levitan (1998b) (also see *Results* [field performance]); sperm availability, see *Results*.

bachiensis has the easiest eggs to fertilize and *S. purpuratus* the most difficult under conditions of sperm limitation. When gametes of these three congeners were released from a syringe in a uniform manner and under similar environmental conditions, no differences in fertilization rates were apparent when spawning was highly synchronous and in close proximity, but when sperm had to travel and disperse for over a minute, *S. purpuratus* had the lowest and *S. droebachiensis* the highest level of fertilization success (Levitan 1998b). The present study indicates that these gamete-level effects can be detected under the natural range of environmental and demographic conditions present in these species.

At the level of individuals, regardless of species averages, encounter frequencies can be highly variable and unpredictable (Fig. 5), so selection for traits that are successful under conditions of sperm limitation or competition are likely to be favored in all three species. Some traits beneficial under sperm limitation may be maladaptive under sperm competition and vice versa. The balance of these opposing trait values may differ across species, dependent on the likelihood and degree of sperm limitation. For eggs the difference may be reflected in the balance between attracting sperm and preventing polyspermy and hybridization. Traits such as egg cell size, the presence and size of accessory structures such as jelly coats, and the production of sperm attractants may be influenced by this balance. For sperm the difference may be reflected in the trade-off between sperm speed and longevity or that between size and number of sperm.

Because female fertilization success depends primarily on the number and proximity of spawning males, it is worth considering why *S. franciscanus* and *S. droebachiensis* do not aggregate as tightly as *S. purpuratus* (Levitan 1998b); high population density entails costs as well as benefits. Intraspecific competition for food results in decreased gamete production at high density (Levitan 1989, 1991, Bureau 1996, Wahle and Peckham 1999). The compensatory nature of gamete production and gamete fertilization can result in largely density-independent zygote production within a species (Levitan 1991, Wahle and Peckham 1999). In the subtidal zone dominated by *S. franciscanus*, food is often

limiting, and gonad size is lower at high density (Bureau 1996). *S. purpuratus* is competitively displaced by *S. franciscanus* and inhabits refuges in wave-exposed shallow subtidal and intertidal habitats (Schroeter 1978). *S. purpuratus* is often crowded into cracks and crevices in these zones, feeding on drift algae (*personal observation*). *S. droebachiensis* is simply rarer than the other species and may have difficulty finding mates. Distribution and abundance of these sea urchins seems to be tied to patterns of recruitment and competitive interactions. Gamete traits may be selected for that maximize reproductive success under these varied conditions.

Historical population densities may have been lower before predatory sea otters were removed by humans (Estes and Palmisano 1974), but killer whales can also quickly reduce sea otter populations, producing a rebounding sea urchin population (Estes et al. 1998). Historical population sizes are therefore difficult to estimate. Still, no evidence suggests that sea otter predation changes the rank order of abundance or local clumping of these species.

The relative importance of biotic and abiotic factors

Abiotic factors as well as population structure may contribute to variation in fertilization success. Under the conditions present in Barkley Sound during the winters and springs of 1995–1999, male distribution and abundance had a larger influence on female fertilization success than did water flow. Advection was a significant factor in two of the three species but only explained between 4% and 6% of the variation in fertilization success. For *S. purpuratus*, high levels of advection reduced fertilization success, probably because sperm were quickly advected away from the relatively small clump of spawning sea urchins. For *S. droebachiensis*, higher levels of advection increased fertilization success, possibly because of the greater spacing between males and females; for this species advection may serve to move sperm from males to females, increasing the chances of fertilization. *S. franciscanus* has intermediate nearest-neighbor distances (Levitan 1998b) that might counteract the positive and negative influences of advection; it also has the largest population sizes and the most homogeneous distribution. In

this species, advection of sperm several meters is less likely to cause a change in local sperm concentration, even at the 25-m² and 100-m² scales investigated in this study.

Different levels of flow near the three species are not likely to cause the differential influence of advection on fertilization. The two species with opposite responses to advection have almost completely overlapping ranges of advection (Fig. 3E). Previous experimental studies under various conditions of flow have noted the negative influence of advection on fertilization (Pennington 1985, Levitan et al. 1992, but see Levitan 1991). These studies were conducted at relatively small spatial scales with only one or a few spawning males.

Effects of surge velocity (turbulence) and water depth could not be detected. High levels of surge in the absence of advection increase the level of turbulence and water mixing but still allow for sperm to slosh back and forth over the spawning females (Denny et al. 1992). Previous theoretical studies (Denny and Shibata 1989) have suggested that increased turbulence will decrease the probability of fertilization by quickly diluting sperm into the sea. This prediction has been confirmed by empirical studies (Levitan 1996a, 1998b, Petersen et al. 2001, but see Coma and Lasker 1997), but the theoretical treatments did not consider large-scale spawning events (Levitan and Young 1995), and the empirical studies were conducted at small spatial scales. Presumably, with regard to the present study, at extremely low and high levels of turbulence, gamete mixing would be too low or too high for eggs and sperm to mix at high enough concentration to allow for fertilization. Although low turbulence did not prevent divers from collecting data, during some situations of high energy, during rough weather, data were not collected. These sea urchins certainly may experience occasional levels of turbulence that would influence fertilization success. Whether they spawn during these extreme weather conditions is unknown. Over the range of flow conditions examined, however, the species generally living under the highest flow conditions, *S. purpuratus*, has the highest level of fertilization. The tight clumping of this species appears to insure fertilization even in a highly turbulent environment.

Water depth has been suggested to influence fertilization success and is one potential reason why many taxa spawn at low slack tide (Levitan 1995). A decrease in the volume of water over spawning animals should increase sperm concentration, but the present study could not detect this effect. Levels of fertilization are weakly negatively correlated with water depth (Fig. 3F), but sea urchins also tend to be denser in shallow water (Table 3). A study of a shallow- and deep-water population of the sea star *Coscinasterias muricata* did find higher levels of fertilization at the shallower site, but the authors attributed the differences to diffusion differences rather than reduced water volume (Babcock

et al. 2000). A more detailed study aimed at this particular effect might be able to tease apart the many confounding population and flow differences as a function of depth. Regardless of whether shallow water directly increases fertilization rates or is simply associated with factors that increase fertilization rates, shallow water may indicate that spawning is likely to be successful and therefore may be a useful cue for spawning.

The Allee effect and abrupt changes in density

A cost of being specialized for a particular set of demographic conditions is that, when conditions change, individuals may be at a disadvantage. For species subject to overexploitation, habitat loss or other anthropogenous or natural disasters may be particularly susceptible to Allee effects (Courchamp et al. 1999, Stephens and Sutherland 1999, Levitan and McGovern, *in press*; Petersen and Levitan, *in press*). The Allee effect, positive density dependence, is a decrease in per capita population growth as density decreases (Allee 1931). Allee effects, at best, result in slowed population recovery and, at worst, negative population growth leading to local or global extinction. The high abundance, which might make a species attractive for exploitation, might also make it more susceptible to Allee effects because adaptations for high density may not be successful after a rapid reduction in density (Levitan and McGovern, *in press*).

Externally fertilizing species with limited mobility may be especially vulnerable to Allee effects, because female fertilization success is highly sensitive to variation in population density (Pennington 1985, Levitan 1991, Levitan et al. 1992, Levitan and Young 1995, Wahle and Peckham 1999). Sperm quickly become diluted in seawater, and female fertilization success diminishes rapidly with distance from a spawning male (Levitan 1995). When sessile or slow moving species spawn out of synchrony or at low density, fertilization rates are generally low (Babcock et al. 1992, Brazeau and Lasker 1992, Oliver and Babcock 1992).

Historically rare species may exhibit spawning traits that alleviate sperm limitation at low population density. These include using density-independent spawning cues such as day length, lunar period, or phytoplankton cues rather than proximate cues such as sperm or physical contact; increased mobility, aggregation, and pair spawning that bring individuals into closer contact; sperm filtering and storage, which increase sperm concentration; hermaphroditism, which increases the probability that a conspecific can function as a mate; and selfing, which eliminates the requirement for a mate (Levitan 1998a). Although phylogenetic constraints may limit the availability of some of these mechanisms, more subtle changes in spawning behavior and the variation in gamete traits, like those described in the present study, may respond to long-term and predictable changes in population density.

The present study indicates that species vary predictably in gamete attributes and performance. One of the most important determinants of female fertilization success is the distribution and abundance of males. Species found at low density have gamete traits that perform well under conditions of sperm limitation, and species found at high density have gamete traits that increase male performance under sperm competition and increase reproductive success of females. These findings suggest a linkage between how gamete traits can influence population dynamics and how population traits can influence selection on gamete traits. Density-dependent selection can be an important structuring force, not only for adults but also for gametes.

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